Trusis, S.N. and A.J.Hilliker. University of Guelph, Ontario, Canada. Analysis of the distribution and homzygous viability of translocation breakpoints with respect to linkage group conservation in D.melanogaster.

Several lines of cytological evidence (Comings 1980; Evans & Filion 1982) argue that, in particular eukaryotic species, at least some portions of the interphase chromosomes (often centromeres and telomeres) are nonrandomly arranged relative to each other. Recently, it has been shown that, at least in one embryonic cell-type in Drosophila, each of the five major chromosome arms is found to occupy a relatively exclusive subvolume or chromosomal domain of the nucleus (Hilliker, manuscript submitted). Each chromosomaldomain appears to be a physically discrete unit consisting of an extensively folded chromosome arm. It is possible that this arrangement is of functional significance.

Indeed, chromosome linkage groups as defined by Drosophila chromosome arms may have remained largely intact during the evolution of the higher Diptera. Sturtevant & Novitski (1941) noted that, chromosome arms were maintained as distinct linkage groups within the (super) genus Drosophila. That is to say, the same genes, as defined by either homologies in polytene chromosome patterns or parallel mutations, were always found associated on the same chromosome arm. In comparing over 14 different species of Drosophila, these chromosome arms maintained their identities, though variability in the linear ordering of the genes within each arm was extensive. A recent study (Foster et al. 1981) shows that linkage group conservation may be a property of the higher Diptera per se. In assaying for biochemically and/or morphologically similar mutations in Lucilia cuprina, and in comparing their linkage relationships to Musca domestica and Drosophila melanogaster, it was discovered that the major linkage groups were conserved. The only major difference found between these Dipterans, which from an evolutionary viewpoint had diverged millions of years prior, was that the linkage groups, as denoted by chromosome arms in D.melanogaster, were actually metacentric chromosomes in L.cuprina and M.domestica. Based on these observations, we proposed that this linkage group conservation might be due to the functional arrangement of the interphase chromosomes.

As a means of testing this hypothesis, a series of reciprocal translocations were synthesized. These translocations altered the established chromosome associations, resulting in the creation of new linkage groups. By assaying for recessive lethality of these induced rearrangements, it might be possible to deduce whether the new type of linkage group created had disrupted any important linkage associations, and thus whether the integrity of the chromosomal region disrupted (not simply that in the immediate vicinity of the breakpoints) was of any functional significance.

Oregon-R males of Drosophila melanogaster, aged 4-5 days, were treated with approximately 2000 rads of gamma irradiation to recover reciprocal translocations between chromosomes 2 and 3. These translocations were analyzed cytologically to determine their polytene chromosome breakpoints, and were subsequently tested for homozygous viability or lethality. Table 1 summarizes these results.

As shown in Table 1, about 53% of 47 analyzed translocations are lethal when homozygous. Earlier studies of induced autosomal 2-3 translocations, similarly obtained by exposing sperm to 2000 rads of irradiation, report slightly higher levels of homozygous lethality. Ytterborn (1970) reported that 66% of 35 synthesized translocations were lethal in the homozygous condition, while Sobels (1972) found that of 84 translocations, 62.2% were homozygous lethal. It is important to note here that, in both cases, no attempt was made to determine the actual rearrangement breakpoints. Since these earlier studies failed to consider the breakpoints or the complexity of the rearrangements, it is possible that the reduced lethality levels we observed could be explained by the fact that the data set presented in Table 1 did not include the majority of complex rearrangements which we obtained. (Upon diagnosis, these stocks were placed aside and never precisely analyzed.)

Analysis of the results presented in Table 1 entailed correlating the homozygous viability or lethality with the actual rearrangement breakpoints. It should be noted though that in analysing the viability of homozygous translocations, the recessive lethality may be attributed to two factors. First, one or both of the actual breakpoints may fall within a vital gene locus. This being the case, the observed lethality would then be a direct result of the breakpoints themselves. On the other hand, it is possible that a proportion of the homozygous lethality may be due to the disruption of a region of the chromosome which requires a linear integrity. Another possibility exists in that new linkage associations may

Table 1. Induced autosomal II-III translocations listed according to polytene chromosome breakpoints and homozygous viability or lethality.

Translocation no.	Chromsome II breakpoint	Chromosome III breakpoint	Viability of translocation homozygote	Status of stock	New stock ID # (if available)
T(2;3)-1	43F1-2	87D4-13	viable	available	T(2;3)-1
T(2:3)-2	2Rh	100F2-5	lethal	lost	
T(2;3)-3	60C2-7	76A2-3	lethal	available	T(2;3)-3
	42A6-19	83D5-E1			
		In(3R)84F12-16;98C3-D1			
T(2;3)-4	50A10-15	90C8-D1	viable	lost	
T(2;3)-5	2Rh	62E3-8	lethal	lost	
T(2;3)-6	60D6-9	94A1-3	lethal	lost	
		In(3R)87D3-10;96F9-11			
T(2;3)-7	57F	70C2-12	lethal	lost	
	het	90C			
T(2;3)-10	44C5-D1	84D3-8	viable	available	T(2;3)-10
T(2;3)-11	36C2-E1	3h	viable	lost	
T(2;3)-13	2Rh	83D	viable	available	T(2;3)-13
T(2;3)-14	53D3-E1	79E2-5	viable	available	T(2;3)-14
T(2;3)-15	47B	92D3-9	viable	available	T(2;3)-15
T(2;3)-16	56D2-E1	67C2-4	viable	lost	
T(2;3)-19	43B1-C1	87D3-E1	viable	available	T(2;3)-19
T(2;3)-20	51D2-7	96E5-9	viable	available	T(2;3)-18
T(2;3)-22	het	het	lethal	lost	
T(2;3)-23	2h	72E2-F1	viable	lost	
T(2;3)-25	42B1-4	82C2-D1	lethal	available	T(2;3)-5
T(2;3)-26	57A2-4	65F2-66A1	lethal	lost	
T(2;3)-27	2Rh	62D6-E2	viable	available	T(2;3)-17
T(2;3)-28	2rh	88C10-E1	viable	lost	
T(2;3)-29	24D1-2	3h	viable	available	T(2;3)-9
T(2;3)-31	38D	69F2-70A1	viable	lost	
T(2;3)-32	21E2-F1	83C2-D1	viable	available	T(2;3)-12
T(2;3)-35	2Rh	98D1-2	lethal	available	T(2;3)-8
T(2;3)-36	33A1-B12	71F2-72A1	viable	lost	
T(2;3)-38	50C11-20	3Lh	viable	lost	
T(2;3)-40	60F1-2	3L(and Y^S?)	lethal	lost	
T(2;3)-42	38A2-C1	89A1-3	viable	lost	
		3Lh			
T(2;3)-43	59C5-D1	3Lh	lethal	available	T(2;3)-16
T(2;3)-44	het	het	lethal	available	T(2;3)-11
T(2;3)-46	38DE	78B	viable	available	T(2;3)-2
T(2;3)-47	24D2-E1	78C	lethal	available	T(2;3)-7
T(2;3)-48	22A1-2	3Rh	lethal	lost	
T(2;3)-49	35B2-B9	3h	viable	lost	
T(2;3)-51	56C3-D1	82E7-8	lethal	lost	
T(2;3)-52	2Lh	99C3-D1	lethal	lost	
T(2;3)-54	36C	3h	viable	lost	
T(2;3)-55	23A	98BC	lethal	lost	
T(2;3)-57	51F	61C7-9	lethal	lost	
T(2;3)-61	23E1-2	64E1-2 (and Y)	lethal	lost	
T(2;3)-63	2h	99D2-4	lethal	lost	
	42A	3h			
	In43F-42A	99D			
T(2;3)-64	24BC	87B4-5	viable	available	T(2;3)-4
T(2;3)-65	59D	64A1-B2	lethal	lost	
T(2;3)-66	21D2-E1	3Lh	viable	lost	
T(2;3)-67	2Rh	99A8-B1	lethal	lost	
T(2;3)-68	21B2-8	82F8-83A1	lethal	available	T(2;3)-6

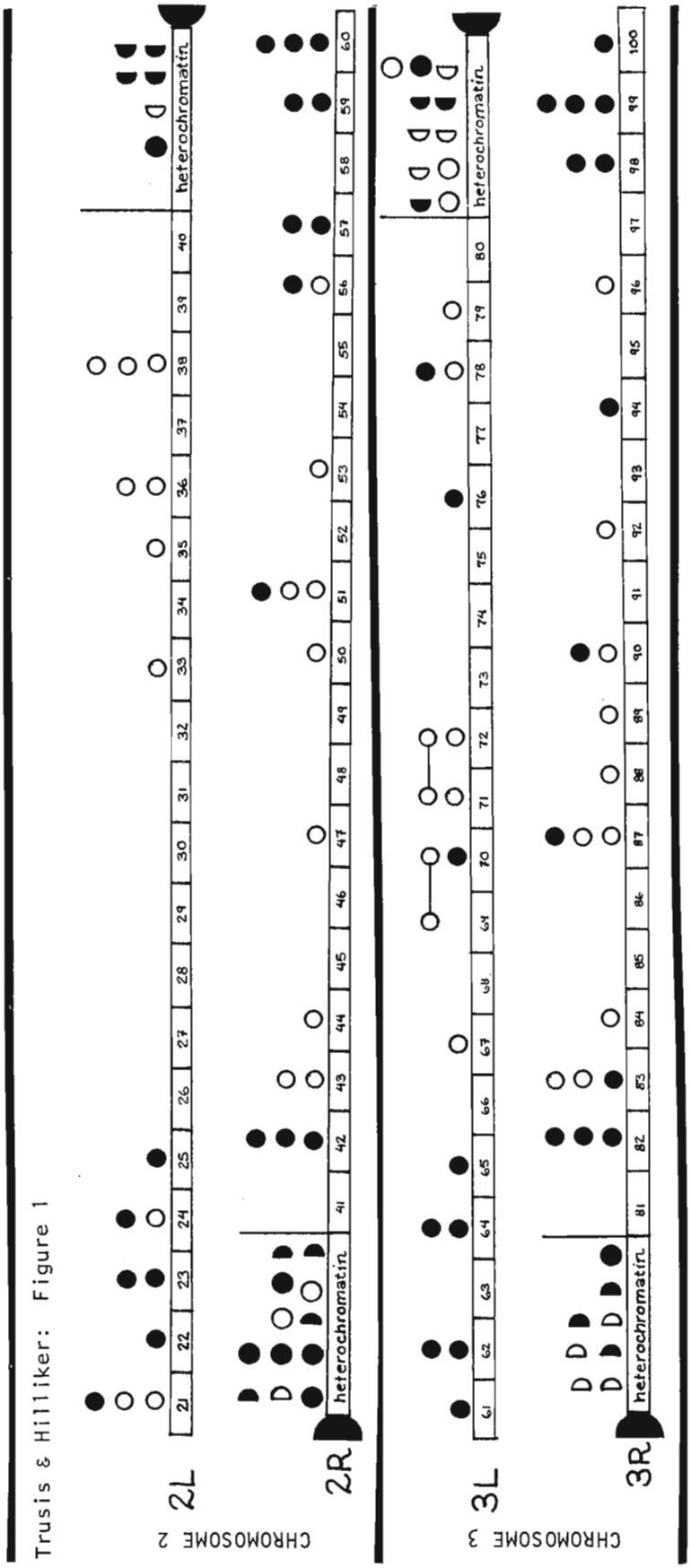

Fig. 1. The distribution of translocation breakpoints with respect to polytene chromosome section indicating homozygous viability (o) or lethality (●). Breakpoints which span more than one polytene section are indicated by o—o. Half circles (◖ or ◗) represent heterochromatic breakpoints which could not be assigned to specific chromosome arms, and are diagrammed as such in both the left and right arm figures. Note also that heterochromatic symbols are arbitrarily placed and do not reflect the relative position of the breaks within heterochromatin.

bring together "new" sets of genes which result in lethal combinations. However, we expect that these latter effects would be dominant lethal and therefore not recoverable.

It was of course necessary to analyse the viability of these translocations in the homozygous condition. Lethal effects would not be evident in the heterozygotes as each translocation heterozygote would still possess one normal homologue for each translocated chromosome, oriented in its appropriate domain. Further, by virtue of somatic pairing, these undisturbed homologues might tend to bring the translocation elements per se into proper alignment with respect to their original linkage groups during interphase.

Figure 1 illustrates the distribution of homozygous lethal and viable breakpoints with respect to each chromosome arm (or domain). Despite the fact that many more translocations are needed for a complete analysis of the genome, Figure 1 documents that the linear integrity of the chromosomal domains can be disrupted quite dramatically. Although this may be simply due to sampling, it appears that the more proximal and distal breakpoints are associated with much higher proportions of homozygous lethality relative to those

Table 2. Inter se complementation crosses tested for viability of trans heterozygotes

Translocation numbers tested

♂	1	3	4	5	6	10	13	14	15	16	19	20	22	23	25	26	28	29	31	32	35	36	42	43	44	46	47	48	49	52	54	55	57	61	64	65	68	♀
1		C					C					C																							C	C		
3				C				C	C			C		C									C			C			C			C						
4		C		C	C		C	C	C						C															C								
5							C	C				C								C			C							C		C	C					
6	NC		C				C																C						C	C								
10	C	C			C		C	C	C			S			C			C				C				C			C					C	C		
13			C	C																										S						C		
14	C	C		C		C								C				C					C			C								C				
15		C					C	C							C				C				C		C		C						C						
16			C																										C									
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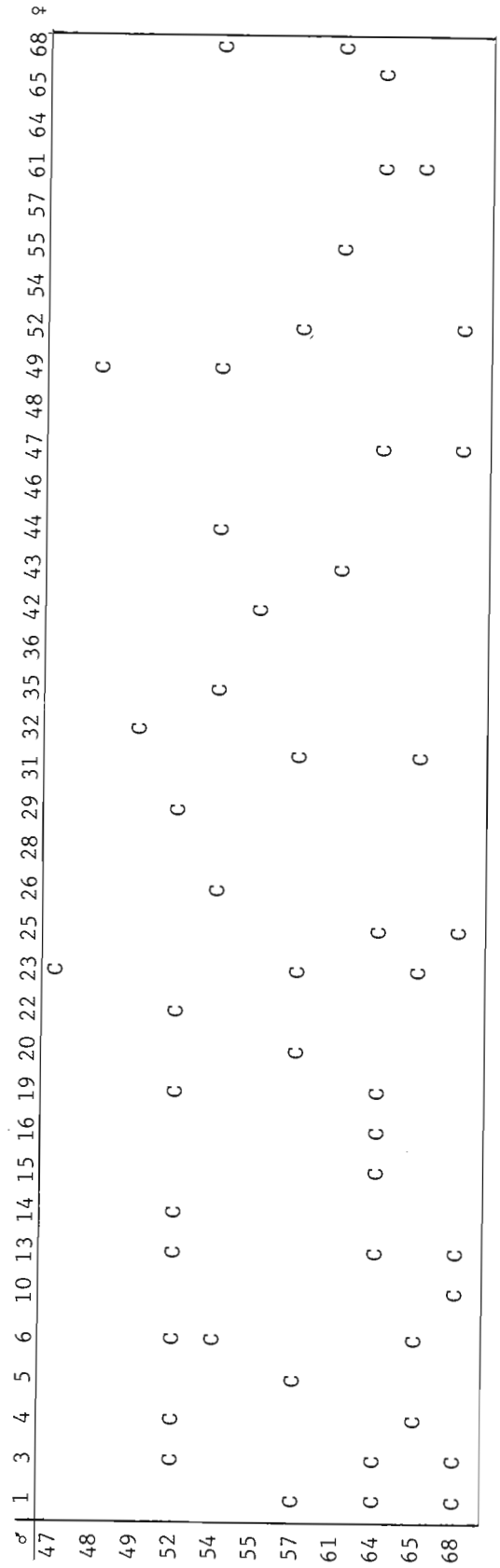
29		C	C					C							C								C				C											
31		C		C										C	C												C											
32																							C				C		C	C	C						
35																										C				C								
36		C	C							C						C							C										C					
42	C		C*		C*	S		C			S							C																				
43	C																																				C	
44		C							C						C												C											
46				C						C	C	C			C	C																		C					

Table 2. Translocation numbers tested (contin.)



to those associated with internal or medial segments.

These findings are contrary to what one might expect, taking into consideration our previous hypothesis regarding linkage group conservation. If maintaining linkage groups is indeed of functional significance, then one might expect that breaks occurring at the telomeres and centromeres would be associated with lower levels of lethality for each respective chromosomal arm since the linkage group would still be largely intact. Similarly, one would expect medial breakpoints to be associated with recessive lethality as they constitute major disruptions of the linkage gorups. Perhaps further data will reveal that the linear integrity of some large sub-regions of the telomeres and possibly the centromeres is important; although the linear integrity need be present in one (normal) chromosome of the translocation heterozygote.

As mentioned earlier, a problem arises in analyzing homozygous lethal translocations, since it is not known whether the lethality is a result of the new linkage associations or merely a function of one or both breakpoints disrupting a vital gene locus. If it happens that the creation of the new linkage groups (i.e., the destruction of the old linkage groups) results in a lethal combination, then two translocations bearing similar yet not identical breakpoints within the disrupted region should also prove to be lethal. To assay this possibility, inter se complementation crosses were carried out between the various analyzed translocations. Stocks of the translocations were balanced and maintained as heterozygotes over TM3. Complementation analysis was subsequently performed by crossing two heterozygous translocation stocks and assaying for surviving trans-heterozygotes. F1 progeny showing none of the dominant phenotypic markers associated with TM3 were presumed to be viable trans-heterozygotes and were therefore said to complement. Table 2 summarizes the results of the initial complementation screen. Unfortunately, the majority of these translocation stocks were lost, thereby limiting the size of the data set presently available.

Despite this limitation, the available data indicates that almost all inter se combinations are viable. (The majority of complementations involved translocations with dissimilar breaks, and might be considered as control crosses.) Although these control crosses do certainly represent the majority of data shown in Table 2, several inter se combinations between related translocations also complemented. Of these combinations, it is interesting to note that the cross involving

a T(2;3)-1, having a break in polytene section 42A6-10, and a T(2;3)-3 ♀, having a break in 43F-1, showed complementation. In referring back to Figure 1, it is evident that this proximal region of 2R euchromatin is associated with a high proportion of homozygous lethal translocations.

Examples of other inter se combinations having similar breakpoints which also complemented include: T(2;3)-5♂ x t(2;3)-13♀ , 2Rh/2Rh ; T(2;3)-10♂ x T(2;3)-3♀, [84D3-8/83D5-E1]; T(2;3)-14♂ x T(2;3)-46♀, [79E2-5/78B]; T(2;3)-42♂ x T(2;3)-1♀, [89A1-3/87D4-13]; T(2;3)-13♂ x T(2;3)-3♀, [83E/83D5-E1]; T(2;3)-57♂ x T(2;3)-20♀, [51F/51D2-7]; T(2;3)-57♂ x T(2;3)-5♀, [61C7-9/62E3-8]; T(2;3)-19♂ x T(2;3)-3♀, [43B1-C1/42A6-19].

Table 2, however, also lists several exceptional combinations which were noted. Four different inter se combinations appeared to be sterile. In each of these combinations, T(2;3)-13♂ x T(2;3)-52♀, T(2;3)-42♂ x T(2;3)-10♀, T(2;3)-42♂ x T(2;3)-19♀, and T(2;3)-10♂ x T(2;3)-20♀, eggs were laid in a dispersed pattern indicating that fertilization had occurred, yet none had appeared to have hatched. It is also interesting to note that one of the reciprocal crosses T(2;3)-13♀ x T(2;3)-52♂ was not sterile and produced progeny according to the expected frequencies. This result was rechecked by repeating each of the reciprocal crosses and again yielded the same results. Two additional inter se combinations involving the crosses T(2;3)-42♂ x T(2;3)-50♀, and T(2;3)-42♂ x T(2;3)-3♀ proved to complement; however, all of the trans-heterozygotes scored (approximately 40 or more in each case) were males. Meiotic segregation per se does not appear to be involved, as each translocation heterozygote male (T(2;3)/bw;ve st e) segregated euploid products (i.e., T(2;3) or t;ttt) in equal proportions. It is possible however that segregation may have been unusual in conjunction with TM3 and/or in translocation heterozygote females. Unfortunately, we can offer no explanation for these results.

The only cross which failed to show complementation was T(2;3)-6♂ x T(2;3)-1♀. In analyzing the significance of this result, it should be noted that only 23 F1 progeny were recovered. Also, upon examining the breakpoints of these translocations, we find that T(2;3)-6 is a complex rearrangement, including an inversion which bears a breakpoint in the same polytene section as T(2;3)-1. It is therefore possible that this lethality, or failure to complement, may be due to common breakpoints within one gene, rather than the type of chromosome configuration produced by this inter se combination. Unfortunately, these exceptions cannot be further analysed since all crosses involve at least one, if not both of the translocations which are no longer available as stocks.

Research is presently ongoing to obtain and analyse additional translocations. This may allow us to identify any important regions of association. Despite the fact that much additional information is still required, a picture is starting to emerge. The results presented here document that many major linkage associations need not be maintained. Some observations, however, suggest that certain regions involving the proximal and distal extremities of each linkage group may require a linear integrity.

A.J.Hilliker was responsible for cytological determination of all polytene chromosome translocation breakpoints. Synthesis and subsequent genetic analysis of the translocations was done by S.N.Trusis.

References: Comings,D.E. 1980, Human Genetics 53:131-143; Evan,K.J. & W.G.Filion 1982, Can.J.Genet.Cytol. 24:583-591; Foster,G.F., M.J.Whitten, C.Konovalov, J.T.A.Arnold & G.Maffi 1981, Genet.Res. 37:55-69; Sobels,F.H. 1972, DIS 48:117; Sturtevant,A.H. & E.Novitski 1941, Genetics 26:517-541; Ytterborn,L. 1970, DIS 45:158.

Ukil, M., K.Chatterjee and A.S.Mukherjee. University of Calcutta, India. Cytophotometric analysis of in situ binding of non-histone protein to the chromatin in Drosophila melanogaster.

The role on non-histone chromosomal protein in the control of gene activity has been reported earlier (Paul & Gilmour 1968; Spelsberg & Hnilica 1969). Since hyperactivity of the X chromosome in Drosophila male is a consequence of relatively higher net transcription of the X chromosome, it is conceivable that the non-histone protein may have a role in mediating the hyperactivation of the X linked genes. Conversely, it may be predicted that non-histone protein may bind differently with X chromosomal DNA sequences in male and female and to substantiate this presumption we carried out the cytophotometric analysis.